NITROGEN METABOLISM OF THE SLIME MOLD DICTYOSTELIUM DISCOIDEUM DURING GROWTH AND MORPHOGENESIS [1]

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Biologists concerned with the biochemistry of developing organisms would profit from analyzing a system in which the processes of growth occur independently from those of morphogenesis. The desirability of such a system has been emphasized by Needham (1942). The slime mold, *Dictyostelium discoideum* Raper, an organism exhibiting this phenomenon in nature, has been described by numerous authors, among them being Raper (1941) and Bonner (1944, 1947). Hirschberg and Rusch (1950, 1951) have indicated the suitability of the slime mold for studies of the biochemistry of development.

In view of current findings, however, there would seem to be no further justification for assuming the morphogenetic phase of development of *D. discoideum* as being devoid of growth processes (Wilson, 1952, 1953). In consequence it may be necessary to exercise caution before associating biochemical and other data with supposed morphogenetic phenomena. However, the mitoses and meioses occur only during particular periods of morphogenesis and not at all under certain conditions (Bonner and Frascella, 1952). Therefore, Bonner and Frascella (1952) suggest that morphogenetic movements are not dependent on mitoses occurring simultaneously.

This investigation is concerned with the nitrogen metabolism occurring in the slime mold and fragments of the slime mold during growth and morphogenesis. It is also the concern of this paper to associate these biochemical changes, insofar as possible, with morphological changes which occur during development of the slime mold.

Methods for Determining μg. N/μg Dry Wt.[3]

The slime molds [4] were cultured according to the method of Bonner (1947). As individual slime mold pseudoplasmodia vary tremendously in size (at least 0.3–1.5 μg. total N) (Gregg, 1950) it was necessary to study nitrogen metabolism changes on the basis of dry weight. In order to weigh samples of slime mold tissue it was necessary to utilize a quartz helical balance having a range of from 1.0–1000.0 μg. The slime molds of various stages and fragments of pseudoplasmodia (Fig. 1) were placed upon tared bits of washed and dried cigarette paper (area approx. 12 sq. mm.). The slime mold tissues were dried in a vacuum desiccator

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TABLE I

Ranges of dry weight and nitrogen determined by analyses of whole and fragments of slime molds during the growth and morphogenetic stages

Type of determination	Vegetative amoebae	Migrating pseudoplasmodia	Mature sorocarps	Spores	Stalks
μg. dry weight	56.2–179.0	70.4–203.0	35.6–127.0	40.5–146.0	24.2–47.6
μg. nitrogen	5.35–18.0	4.40–20.8	3.03–11.6	3.38–14.6	0.38–2.96

at room temperature or in a drying oven at 60° C. for approximately twelve hours. The samples were then weighed on the quartz helical balance (Table I). The weighed samples of slime mold tissues were then analyzed for total N (TN). The magnitude of the dry weight and total nitrogen of the samples is shown in Table I.

The nitrogen determinations were made by a modification of the method of Bruel *et al.* (1946). The results were expressed as μg N/μg dry wt. Controls were conducted during the experiments by analyzing cigarette paper for the presence of traces of nitrogen.

GROWTH PHASE

Vegetative amoebae: Figure 1

The vegetative amoebae were prepared for analysis by the following steps:

1. Harvested from four Petri dishes by rubbing the agar surfaces with a glass rod in the presence of approximately 10 ml. of distilled H_2O. Amoebae and H_2O were filtered through a small bit of cotton to remove agar particles.

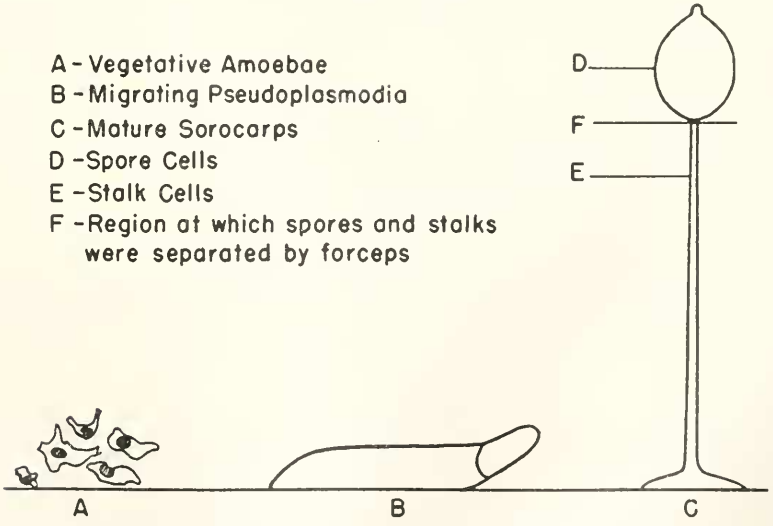

FIGURE 1. Diagram of the growth and morphogenetic stages of the slime mold, *D. discoideum*, used in the analyses.

2. Amoebae centrifuged 5–10 minutes in a 15-ml. centrifuge tube at approximately 800 g.

3. Supernatant removed and discarded. The amoebae and *E. coli* at bottom of tube placed on surface of 10 ml. of 0.95 *M* sucrose solution in a 15-ml. centrifuge tube. Material centrifuged 5 minutes at approximately 500 g.

4. Supernatant removed and discarded. Amoebae placed in fresh tube of 0.95 *M* sucrose and centrifuged 5 minutes at approximately 500 g.

5. Supernatant removed and discarded. Amoebae suspended in distilled H_2O and recentrifuged 5 minutes at approximately 800 g.

6. Supernatant discarded and slime molds transferred to tared cigarette papers for drying, weighing and nitrogen analyses. The transfer was made with a thin-walled Pyrex glass pipette approximately 0.5 mm. I.D. at the tip.

Centrifugation of the slime mold amoebae and *E. coli* in 0.95 *M* sucrose tends to decrease the number of bacteria which are thrown to the bottom of the centrifuge tube. Upon microscopic examination of such preparations, very few bacteria were seen. It is believed that the relatively low numbers of bacteria present eliminate the possibility of significant interference by extraneous nitrogen in the analyses of the vegetative amoebae.

Vegetative amoebae subjected to the 0.95 *M* sucrose washing treatment, as described in steps 1–5, when placed on an agar surface aggregated and produced normal-appearing mature sorocarps.

Morphogenetic Phase

Migrating pseudoplasmodia: Figure 1

Between ten and twenty migrating pseudoplasmodia were transferred from the agar surface to the tared cigarette papers with a hair loop. The tissues were dried, weighed and analyzed for total nitrogen (TN).

Mature sorocarps: Figure 1

Between fifteen and twenty-five mature sorocarps were transferred from the agar surface to the tared cigarette papers with fine-tipped forceps. They were dried, weighed and analyzed for total nitrogen (TN).

Spores and stalks: Figure 1

The mature sorocarps were separated into spores and stalks with fine-tipped forceps and transferred to tared cigarette papers. They were dried, weighed and analyzed for total nitrogen (TN).

Results

Total nitrogen (TN). Table II

The migrating pseudoplasmodia contain slightly less TN relative to their dry weight than the vegetative amoebae. This 9.13% loss is not statistically significant ($P > 0.05$).[5]

However, during the transition from the migrating pseudoplasmodia to the

[5] P values in this investigation were calculated by Student's t-test.

mature sorocarps, the TN/dry wt. ratio decreases 14.6% relative to that of the migrating pseudoplasmodia. Thus the mature sorocarps have been shown to contain significantly less TN/dry wt. than the migrating pseudoplasmodia ($P < 0.01$).

Analyses of the spores and stalks separately have shown that the observed loss of TN/dry wt. from the mature sorocarp did not come from the spores during

TABLE II

Analyses of nitrogen components of the vegetative and morphogenetic stages expressed as μg N/dry weight. These ratios represent the actual values with their standard deviations × 10^2. The dry weight values necessary in determining the final values of the various components other than total nitrogen were calculated from the total nitrogen/dry weight data. The numerals in parentheses refer to the number of experiments performed.

Nitrogen component	Vegetative amoebae	Migrating pseudoplasmodia	Mature sorocarps	Spores	Stalks
TN	10.30 ±0.691 (8)	9.36±1.52 (14)	7.99±0.650 (14)	9.59±2.94 (11)	4.73 ±1.55 (11)
TEN	5.70 ±2.47 (7)	6.81±0.730 (4)	4.49±0.976 (4)	6.20±2.80 (5	3.30 ±1.33 (4)
TEPN	4.75 ±2.61 (7)	5.66±0.260 (4)	2.81±0.957 (4)	2.63±0.656 (5)	0.987±0.333 (4)
TNPN	0.999±0.315 (7)	1.36±0.575 (4)	2.31±0.784 (4)	1.86±0.469 (5)	1.71 ±0.212 (4)
TUN	5.22 ±2.54 (7)	3.77±0.409 (4)	3.61±0.735 (4)	4.83±1.04 (5)	2.00 ±0.684 (4)
TUN+TEPN	9.97 ±1.46 (7)	9.43±0.641 (4)	6.42±0.509 (4)	7.46±1.06 (5)	2.98 ±0.776 (4)

TN = Total nitrogen
TEN = Total extractable nitrogen
TEPN = Total extractable protein nitrogen
TNPN = Total non-protein nitrogen
TUN = Total un-extractable nitrogen
TUN + TEPN = Total un-extractable nitrogen + total extractable protein nitrogen

culmination, since actually a slight increase (2.46%) occurred in the TN/dry wt. ratio of the spores, relative to that of the migrating pseudoplasmodia. This difference was not statistically significant ($P > 0.7$).

The stalks, however, were shown to have lost a statistically significant amount of TN/dry wt. (49.5%) relative to the migrating pseudoplasmodia ($P < 0.001$).

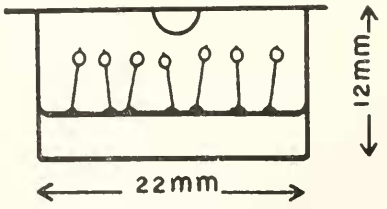

FIGURE 2. Diffusion cell with hanging drop of 0.01 N H_2SO_4 to absorb NH_3 liberated by the slime molds.

The loss of TN/dry wt. which was detected in the analyses of mature sorocarps relative to the migrating pseudoplasmodia may be attributed to an excretion of ammonia. The fact that ammonia is excreted by the slime mold *D. discoideum* in detectable quantities was determined by two methods.

The first method consisted in placing approximately twenty-four migrating pseudoplasmodia on non-nutrient or nutrient agar in a small Conway type vessel

TABLE III

Nitrogen (NH_3) excreted by D. discoideum *during the transition from migrating pseudoplasmodia to mature sorocarps determined by titration*

Mean no. of slime molds per diffusion cell	μg. N	μg. N—control	No. of experiments performed
24	—	0.545 ± 0.0153	3
0 (control)	0.009	—	1

(Fig. 2). A drop (11.8 μl.) of 0.01 N H_2SO_4 was placed on a paraffined glass slide, inverted and sealed to the vessel by means of a paraffin-vaseline mixture or Lubriseal stopcock grease. The slime molds were allowed to culminate completely. The drop of 0.01 N H_2SO_4 was then titrated to a methyl red end-point with a micro burette containing 0.01 *M* NaOH (Gregg, 1950) (Table III).

The second method was conducted similarly to the first with the exception that approximately 2.0 μl. of Nessler's reagent was added to the acid drop rather than titrating it. Nessler's reagent gave a strong positive reaction, demonstrating the presence of ammonia in the acid drop. Controls involving Nessler's reagent gave very weak positive results relative to the experimentals. These experiments have shown beyond doubt that ammonia is lost from the organism during certain of its developmental processes.

With a view to detecting possible changes in nitrogenous components which might be masked if total nitrogen analyses alone were considered, the various stages and fragments of the slime molds were fractionated in the manner described in Table IV. The fractions were designated as total extractable nitrogen, total extractable protein nitrogen, total non-protein nitrogen, total un-extractable nitrogen and total un-extractable nitrogen + total extractable protein nitrogen.

TABLE IV

Scheme designating source of various nitrogen components of slime mold tissues analyzed by micro-Kjeldahl procedure. (Modified from Gregg and Ballantine, 1946)

Slime mold tissues

↓

Homogenize in extractant composed of 0.65% NaCl in 0.01 *M* phosphate buffer at pH 7.2 and dilute to 150 μl. with extractant

↓

Remove 20–30 μl. for total nitrogen (TN)

↓

Centrifuge remainder 15 minutes at 1000 g in Misco Air turbine

Particulate matter analyzed for total unextractable nitrogen (TUN)	Remove 30 μl. of supernatant for analysis of total extractable nitrogen (TEN)	Remove 80 μl. of supernatant. Deproteinize with 15 μl. of 0.67 H_2SO_4 followed by 8 μl. of 10% Na_2WO_4. Centrifuge 15 minutes at 1000 g in Misco air turbine. Analyze precipitate for total extractable protein nitrogen (TEPN). Analyze entire supernatant for total non-protein nitrogen (TNPN).

The slime molds were harvested from the agar surfaces by the techniques described previously (Steps 1–5) with the exception that the preparations were homogenized immediately on the ground glass surface of a micro-homogenizer (Fig. 3) rather than being subjected to a weighing procedure on the quartz helical balance. The magnitude of the nitrogen present in each of the five components is listed in Table V.

Total extractable nitrogen (TEN). Table II

The 12.0% increase in TEN/dry wt. of the migrating pseudoplasmodia was not shown to be statistically significant relative to that of the vegetative amoebae ($P > 0.3$). The TEN/dry wt. content of the mature sorocarps exhibited a 34.1% decrease from that of the migrating pseudoplasmodia. This difference is statistically significant ($P < 0.01$).

From the examination of the TEN/dry wt. values of the spores and stalks it may be seen that a 51.5% loss from the stalks constitutes the major part of the decrease noted in the mature sorocarps. This difference is statistically significant

TABLE V

Ranges of nitrogen in various components determined by analyses of whole and fragments of the slime molds during the growth and morphogenetic stages

Nitrogen component analyzed	Vegetative amoebae	Migrating pseudoplasmodia	Mature sorocarps	Spores	Stalks
μg. TN	4.29–18.1	2.21–10.0	0.85–3.33	1.02–3.37	0.34–0.82
μg. TEN	1.43–19.8	4.13–12.1	2.28–4.69	0.89–5.87	0.27–0.75
μg. TEPN	2.59–47.1	11.0–20.9	2.89–6.49	0.89–3.26	0.27–0.75
μg. TNPN	1.45–5.50	2.0–6.33	1.60–6.36	0.57–2.46	0.56–1.11
μg. TUN	17.7–37.7	12.3–25.0	4.21–21.6	3.62–10.9	1.23–1.63

($P < 0.01$) while the 8.96% difference in TEN/dry wt. between the spores and migrating pseudoplasmodia was not statistically significant ($P > 0.6$).

Total extractable protein nitrogen (TEPN). Table II

The 11.9% increase in TEPN/dry wt. which the migrating pseudoplasmodia exhibit relative to that of the vegetative amoebae is not statistically significant ($P > 0.4$). The mature sorocarps, however, show a significant decrease of 50.4% of their TEPN/dry wt. when compared to that of the migrating pseudoplasmodia ($P < 0.01$). The decrease occurs from both the spores and the stalks. Values of other nitrogen components of the spores, specifically, TN/dry wt., TEN/dry wt., and TUN/dry wt., suggested that little nitrogen utilization occurs in that region. The 53.5% decrease of the spore TEPN/dry wt. from that of the migrating pseudoplasmodia is statistically significant ($P < 0.001$) and is indicative of protein metabolism within the spores which has been masked in previous analyses by the presence of other nitrogenous components, namely TNPN/dry wt. and TUN/dry wt. The stalks show a decrease of TEPN/dry wt. amounting to 82.5% of the initial amount present in the migrating pseudoplasmodia. This difference is statistically significant ($P < 0.001$).

Total extractable non-protein nitrogen (*TNPN*). Table II

The value of the TNPN/dry wt. of the migrating pseudoplasmodia, although 36.1% greater than that of the vegetative amoebae, is not significantly different ($P > 0.1$). The mature sorocarps show a 69.9% greater TNPN/dry wt. content than the migrating pseudoplasmodia. Statistical analysis, however, fails to confirm what appears to be a significant difference ($P > 0.05$). The spores and stalks show increases relative to the migrating pseudoplasmodia of 36.8% and 25.7%, respectively. Neither of these increases is significantly different from the migrating pseudoplasmodia ($P > 0.1$ and $P > 0.2$). It is possible to compute the entire amount of non-protein nitrogen produced by making the assumption that the losses observed between certain of the TN/dry wt. values of the various stages resulted from an excretion of non-protein nitrogen. These values, in addition to the re-

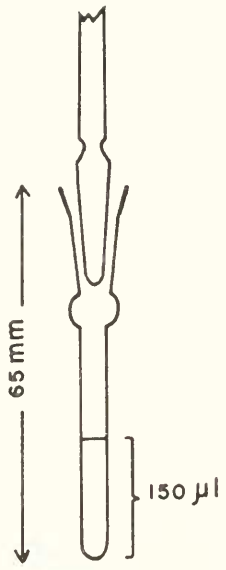

FIGURE 3. Micro-homogenizer constructed from a 5/20 Pyrex interconnecting joint.

tained non-protein nitrogen values, comprise the entire quantity of non-protein nitrogen produced. Figure 4 demonstrates that the observed increase of the entire TNPN/dry wt. during development may be attributed to a quantitatively similar utilization of the TEPN + TUN/dry wt. component. The greatest discrepancy is found at the migrating stage since the level of TNPN/dry wt. is in excess of that which can be accounted for by a breakdown of the TEPN + TUN/dry wt. component. The excess TNPN/dry wt. can possibly be attributed to digestion of ingested *E. coli* with consequent production of non-protein nitrogen.

Total un-extractable nitrogen (*TUN*). Table II

The TUN/dry wt. content of the migrating pseudoplasmodia shows a decrease of 27.8% relative to the vegetative amoebae. This decrease is not statistically significant ($P > 0.2$). The mature sorocarps show a 4.24% decrease as compared to the migrating pseudoplasmodia. Neither this decrease nor the 28.1% increase

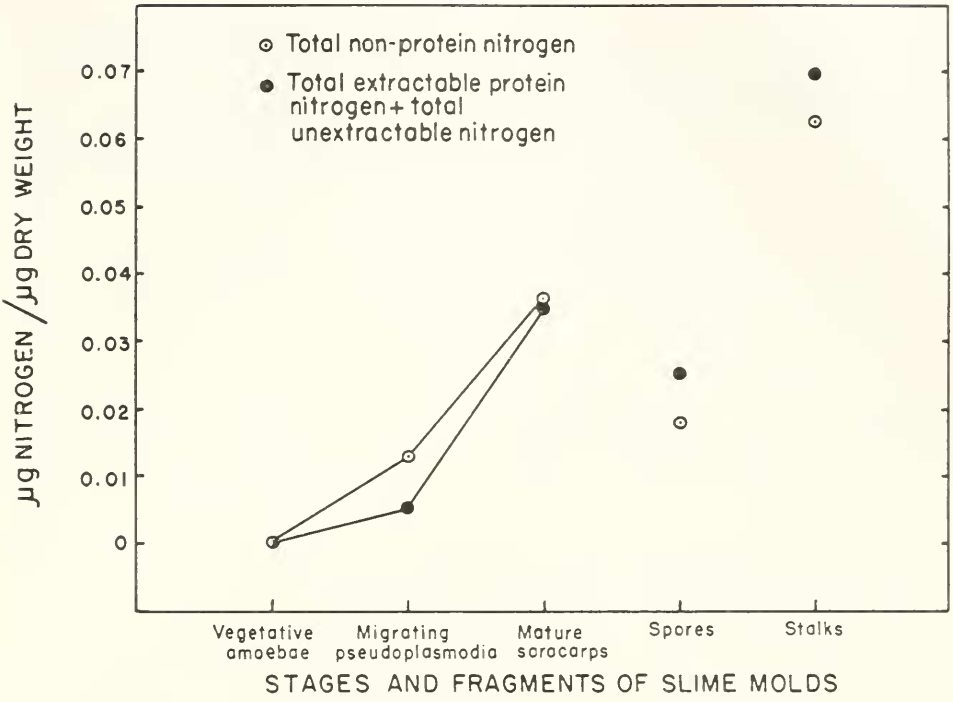

FIGURE 4. Graph relating the accumulated quantity of the TEPN + TUN/dry wt. component metabolized during development with the consequent increase in TNPN/dry wt. The accumulated TNPN/dry wt. was computed from the values of the retained TNPN/dry wt. plus that excreted from the organism as calculated from the decreases in TN/dry wt.

in the TUN/dry wt. component of the spores differs significantly from the value of the migrating pseudoplasmodia ($P > 0.7$ and $P > 0.05$). The slight but insignificant decrease in TUN/dry wt. indicated in the mature sorocarps results from the 46.9% decrease of TUN/dry wt. which occurs in the stalks. Relative to the migrating pseudoplasmodia this decrease has been shown to be statistically significant ($P < 0.01$). It appears as though this component is utilized by the stalks as well as the TEPN/dry wt. during the culmination of the slime mold.

Total extractable protein nitrogen + total un-extractable nitrogen (TEPN + TUN). Table II

When the sum of the two components TEPN/dry wt. and TUN/dry wt. was calculated it was found that the migrating pseudoplasmodia do not show a statistically significant decrease (5.42%) relative to the vegetative amoebae ($P > 0.5$). During culmination, however, a 31.9% decrease in the component TEPN + TUN/dry wt. occurred in comparison with the migrating pseudoplasmodia. This difference is statistically significant ($P < 0.001$). Both the spores and the stalks contributed to this decrease noted in the mature sorocarps. The decreases of the spores and stalks from the migrating pseudoplasmodia amounted to 20.7% and 68.4%, respectively. These differences are statistically significant ($P < 0.02$ and $P < 0.001$).

Discussion

Nitrogen changes occurring during the transition from the vegetative amoebae to the migrating pseudoplasmodia

The levels of the various nitrogenous components, total nitrogen/dry wt., total extractable nitrogen/dry wt., total extractable protein nitrogen/dry wt., total non-protein nitrogen/dry wt. and total un-extractable nitrogen/dry wt. of the vegetative amoebae and the migrating pseudoplasmodia were investigated. These values indicate that statistically significant nitrogen changes do not occur during the transition from the vegetative amoebae to the migrating pseudoplasmodia.

Nitrogen changes occurring during the transition from the migrating pseudoplasmodia to the mature sorocarps

The total nitrogen/dry wt. shows a statistically significant decrease during the transition from the migrating pseudoplasmodia to the mature sorocarps. The assumption has been made that this decrease reflects a loss of nitrogen resulting from the excretion of ammonia. The excretion of ammonia during this period has been demonstrated.

By a fractionation procedure it was possible to demonstrate that this loss of total nitrogen/dry wt. results from the metabolism of the total extractable protein nitrogen/dry wt. component. The increase in total non-protein nitrogen/dry wt. resulting from nitrogen metabolism during this period has been found to be quantitatively equivalent to the decrease noted in the total extractable protein nitrogen + total un-extractable nitrogen/dry wt. component (Fig. 4).

It was of interest to determine in which region of the pseudoplasmodium particular nitrogenous components were being utilized during the transition from the migrating stage to the mature sorocarp. In order that this might be accomplished, the mature sorocarps were separated into their major morphological entities, the spores and stalks. In analyzing the spores it was found that no loss of total nitrogen/dry wt. occurs. Therefore, the loss of total nitrogen/dry wt. observed in the intact mature sorocarps can be attributed to a decrease of total nitrogen/dry wt. in the stalks. The nitrogenous components responsible for the decrease of total nitrogen/dry wt. in the stalks were shown to be total extractable protein nitrogen/dry wt. and total un-extractable nitrogen/dry wt. The spores, too, show a decrease in total extractable protein nitrogen/dry wt., although of a smaller magnitude than that of the stalks. The spores do not show a loss in total nitrogen/dry wt. as simultaneous increases of other nitrogenous components are sufficient to mask the loss of total extractable protein nitrogen/dry wt.

From these data it is reasonable to suggest that both pre-spores and pre-stalks utilize proteins during the culmination process of morphogenesis. Since the pre-stalk cells are primarily responsible for raising the spore mass (Bonner, 1944; Raper and Fennell, 1952) it is not surprising that the metabolic requirements of the stalk-forming cells differ somewhat from those of the spore cells. During culmination the stalk cells build a cellulose sheath around themselves and cellulose is deposited in the walls of the spore cells (Raper and Fennell, 1952). It is suggested that the slime mold probably does not carry sufficient carbohydrate reserves both to synthesize cellulose and to utilize for purposes of obtaining energy. In this event proteins could be converted into cellulose or used for energy production.

While it is difficult to establish for which of these events protein metabolism is taking place, it is interesting to point out that the major nitrogen changes occur during the culmination process, at which time the spore and stalk cells are formed. While these data are suggestive of a relationship between protein metabolism and cellulose synthesis the hypothesis does not preclude the activity of other intrinsic mechanisms dependent upon protein breakdown.

Summary

1. Equipment and procedures incidental to determining the nitrogen metabolism of the vegetative amoebae, whole pseudoplasmodia, and fragments of pseudoplasmodia of the slime mold *Dictyostelium discoideum* during growth and morphogenesis have been described.

2. During the transition from the vegetative amoebae to the migrating pseudoplasmodia no statistically significant changes were found in any of the nitrogenous components under investigation.

3. During the transition from the migrating pseudoplasmodia to the mature sorocarps statistically significant decreases were found in certain nitrogenous components.

4. By analyzing spores and stalks separately it was possible to attribute nitrogen changes occurring in the intact mature sorocarps to particular regions.

5. The excretion of ammonia during the transition from the migrating pseudoplasmodia to the mature sorocarps was demonstrated.

6. The relationship between nitrogen metabolism and the synthesis of cellulose was discussed.

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